PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference VTT 96 PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FI00/00707	International filing date *(day/month/year)* 21.08.2000	Priority date *(day/month/year)* 20.08.1999
International Patent Classification (IPC) or national classification and IPC7 C 12 N 1/14, C 12 N 15/80 //C 12 N 1/15		
Applicant VALTION TEKNILLINEN TUTKIMUSKESKUS et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13.03.2001	14.11.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Yvonne Siösteen/EÖ Telephone No. 08-782 25 00

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI00/00707

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-36 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under article 19
pages _____ , filed with the demand
pages 37-40 , filed with the letter of 15.10.2001

☒ the drawings:
pages 1-16 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages 1-4 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-31	YES
	Claims		NO
Inventive step (IS)	Claims	3, 17, 25, 30	YES
	Claims	1, 2, 4-16, 18-24, 31	NO
Industrial applicability (IA)	Claims	1-31	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The examination report is based on the amended claims of 15 october 2001.

The claimed invention relates to a method for producing a product by cultivating a microorganism. A common problem when fermenting is that foam formation can limit the formation of the product. Therefore a purpose with the present invention is to decrease the foam formation during cultivation of a microorganism. The finding that hydrophobins are responsible for foam formation has led to new production strains. The fungus trichoderma is genetically modified so that it does not produce an essential amount of the hydrophobins HFB I and/or HFB II .

A method for reducing foam formation when cultivating Bacillus in order to achieve a better production of polypeptides is disclosed in WO 9822598. The Bacillus is mutated in the gene coding for surfactin, which is a cyclic lipopeptide responsible for foam formation when culturing Bacillus. The mutated strain produces less surfactin than the non mutated strain.

However, it has not been disclosed in the prior art that hydrophobins, could be responsible for foam formation during cultivation of a microorganism. Neither has a method for decreasing foam formation by modifying the gene responsible for hydrophobin production been disclosed. It was unexpected that hydrophobins which are not lipopeptides or lipoproteins were responsible for foam production. Therefore, claims 3, 17,25 and 30 are novel and considered to involve an inventive step.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

Claim 1-2, 4-16, 18-24 and 31 do not disclose the invention in a sufficiently concise manner. They should be restricted to the alleged inventive feature being that the proteins are hydrophobins. The expression "hydrophobin-like molecules" in claim 2 and 14 is not clear and concise. Thus, claims 1-2, 4-16, 18-24 and 31 lack inventive step.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The expression "hydrophobin like molecules" of claims 2 and 14 is indefinite. Therefore, claims 2 and 14 do not fulfil the requirements of clarity and conciseness according to PCT Rule 6.1(9).

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CLAIMS

1. A method for decreasing the foam formation during cultivation of a microorganism, characterized in that the process comprises the steps of

- modifying the microorganism in such a way that the microorganism does not produce an essential amount of at least one of the proteins, polypeptides or peptides associated with foam formation during cultivation, said proteins, polypeptides or peptides being amphipathic or hydrophobic proteins, polypeptides or peptides, not including lipopeptides or lipoproteins ; and
- cultivating the microorganism under suitable culture conditions.

2. The method of claim 1, characterized in that the proteins, polypeptides or peptides associated with foam formation are hydrophobins or hydrophobin-like molecules.

3. The method of claim 1 or 2, characterized in that the hydrophobins are HFB I and/or HFBII of *Trichoderma*.

4. The method of any one of claims 1 to 3, characterized in that the modification comprises genetic modification of the microorganism.

5. The method of claim 4, characterized in that the genetic modification comprises genetic modification of a DNA sequence encoding a protein, polypeptide or peptide regulating the production of at least one of the proteins, polypeptides or peptides associated with foam formation.

6. The method of claim 4, characterized in that the genetic modification comprises genetic mofication of the regulatory region of a gene encoding at least one of the proteins, polypeptides or peptides associated with foam formation

7. The method of claim 4, characterized in that the genetic modification comprises genetic modification of a DNA sequence encoding at least one of the proteins, polypeptides or peptides associated with foam formation.

8. The method of claim 7, characterized in that the genetic modification comprises inactivation of a DNA sequence encoding at least one of the proteins, polypeptides or peptides associated with foam formation.

9. The method of claim 8, characterized in that the genetic modification comprises deletion of a DNA sequence encoding at least one of the proteins or polypeptides or peptides associated with foam formation.

10. A method for producing a product by cultivating a microorganism, characterized in that the process comprises the steps of
- modifying the microorganism in such a way that the microorganism does not produce an essential amount of at least one of the proteins, polypeptides or peptides associated with foam formation during cultivation, said proteins, polypeptides or peptides being amphipathic or hydrophobic proteins, polypeptides or peptides, not including lipopeptides or lipoproteins;
- cultivating the microorganism under suitable culture conditions; and
- recovering the product from the cultivation.

11. The method of claim 10, characterized in that the product is a protein or a metabolite or biomass.

12. The method of claim 10, characterized in that the product is a recombinant product.

13. A production host strain, characterized in that the host strain is genetically modified not to produce an essential amount of at least one of the amphipathic or hydrophobic proteins, polypeptides or peptides associated with foam formation during cultivation of the non-modified production host strain, said proteins, polypeptides or peptides being amphipathic or hydrophobic proteins, polypeptides or peptides, not including lipopeptides or lipoproteins.

14. The production host strain of claim 13, characterized in that the proteins, polypeptides or peptides associated with foam formation are hydrophobins or hydrophobin-like proteins.

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15. The production host strain of claim 13 or 14, c h a r a c t e r i z e d in that the strain is a fungal strain.

16. The production host strain of claim 15, c h a r a c t e r i z e d in that the host strain is a *Trichoderma* strain.

17. The host strain of claim 16, c h a r a c t e r i z e d in that the proteins are HFB I or HFB II or both of *Trichoderma*.

18. The host strain of claim 13 or 14, c h a r a c t e r i z e d in that the host srain is a bacterial strain.

19. The host strain of claim 18, c h a r a c t e r i z e d in that the strain is a *Bacillus spp.* strain, a *Streptomyces spp.* strain or an *E. coli* strain .

20. A production host strain, c h a r a c t e r i z e d in that the host strain is
- genetically modified not to produce an essential amount of at least one of the proteins, polypeptides or peptides associated with foam formation during cultivation of the non-modified production host strain, said proteins, polypeptides or peptides being amphipathic or hydrophobic proteins, polypeptides or peptides, not including lipopeptides or lipoproteins; and is
- modified to be capable of producing a product of interest.

21. A production host strain, c h a r a c t e r i z e d in that the host strain is genetically modified not to produce an essential amount of at least one of amphipathic or hydrophobic proteins, polypeptides or peptides, said proteins, polypeptides or peptides being amphipathic or hydrophobic proteins, polypeptides or peptides, not including lipopeptides or lipoproteins, and has an increased capability to produce a product of interest.

22. The host strain of claim 21, c h a r a c t e r i z e d in that the host strain is modified to be capable of producing a product of interest.

23. The host strain of any one of claims 20 to 22, c h a r a c t e r i z e d in that the host strain is a fungal strain.

24. The host strain of any one of claims 20 to 23, c h a r a c t e r i z e d in that the host strain is a *Trichoderma* strain.

25. The host strain of any one of claims 20 to 24, c h a r a c t e r i z e d in that the hydrofobins are HFB I or HFB II of *Trichoderma*.

26. The host strain of any one of claims 20 to 22, c h a r a c t e r i z e d in that the microorganism strain is a bacterial strain.

27. The host strain of claim 26, c h a r a c t e r i z e d in that the microorganism strain is a *Bacillus spp.* strain, a *Streptomyces spp.* strain or an *E. coli* strain .

28. The host strain of any one of claims 20 to 27, c h a r a c t e r i z e d in that the product of interest is a protein or a metabolite or biomass.

29. The host strain of any one of claims 20 to 27, c h a r a c t e r i z e d in that the product of interest is a recombinant product.

30. The host strain of any one of claims 20 to 29, c h a r a c t e r i z e d in that the host strain is genetically modified to be capable of producing a fusion molecule comprising a molecule of interest fused to a hydrophobin.

31. A process for producing an enhanced amount of a product of interest, c h a r a c - t e r i z e d in that the process comprises the steps of

- cultivating a production host strain of any one of claims 20 to 30; and
- recovering the product from the cultivation.